matic enrichment of the POI. Using AptA-MS several known and novel interactors of human Heat Shock Factor 1 (HSF1) tagged with GFP have been identified, some of which showed an increased association following heat shock (HS). In addition, post-translational modifications (PTMs) of HSF1 and the co-precipitated histones have been identified without additional tailored enrichment steps for these modifications. AptA-MS has also been applied with other aptamers (e.g. NELF-aptamer) (Pagano et al., "Defining NELF-E RNA Binding in HIV-1 and Promoter-proximal Pause Regions," *PLoS Genet.* 10:e1004090 (2014), which is hereby incorporated by reference in its entirety) to enrich its target from *Drosophila* S2 cells. The results indicate that in addition to purifying transiently transfected HSF1-GFP from human cells, the GFP-aptamer is capable of enriching endogenous GFP-tagged RNA polymerase II (Pol II) from yeast, as well as formaldehyde crosslinked GFP from *Drosophila* S2 cells, thereby making it a versatile tool for affinity purification of GFP-tagged proteins from various sources.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. **1** shows the AptA-MS workflow. The polyadenylated GFP-aptamer is annealed to desthiobiotin (dB) labeled oligo dT and immobilized on streptavidin (SA) coated magnetic Dynabeads. Cellular lysate containing Protein of interest (POI)-GFP is incubated with the immobilized aptamer beads that are washed and finally eluted with biotin. Eluate is subjected to MS, and the data is processed through a pipeline for protein identification followed by enrichment analysis, interaction score quantification, and PTM analysis. Figure partially created with BioRender.com using the GFP structure (PDB ID: 4KW4, Barnard et al., "Crystal Structure of Green Fluorescent Protein," doi: 10.2210/pdb4KW4/pdb (2014), which is hereby incorporated by reference in its entirety).

[0010] FIGS. **2**A-**2**D show enrichment of GFP-tagged proteins by AptA-MS.

[0011] FIG. **2**A is a schematic representation of the experimental design. FIG. **2**B shows cellular lysates prepared from HCT116 cells transfected with GFP or HSF1-GFP expressing plasmids that were analyzed by anti-GFP (green) and anti-Actin (red, loading control) western blot. GFP (Abcam, ab290) and Actin (Sigma, MAB1501) antibodies were used at 1:2000 and 1:5000 dilutions, respectively. FIG. **2**C shows lysate from cells expressing GFP or HSF1-GFP were precipitated with the GFP- or Control (Ctrl)-aptamer and eluates were analyzed by gel electrophoresis and silver-staining. The bottom panel shows a fluorescence image of the eluates. FIG. **2**D shows enrichment analysis of HSF1 in AptA-MS samples from cells expressing GFP or HSF1-GFP, before or after heat shock, pulled-down with the GFP- or the control-aptamer. Plot represents data from five independent biological replicates.

[0012] FIGS. **3**A-**3**B show a comparison of aptamer vs antibody affinity purification. FIG. **3**A shows cellular lysates prepared from HCT116 cells that were transfected with HSF1-GFP expressing plasmids and were affinity-purified by the GFP-aptamer (Apt) or the GFP-antibody (Ab). Eluates were run after RNase treatment on an SDS-PAGE and visualized by silver-stain. FIG. **3**B shows comparative analysis of AptA-MS vs. conventional immunoprecipitation. Identified proteins from HSF1-GFP AptA-MS or CP.RTP immunoprecipitation (DeBlasio et al., "Insights into the polerovirus-plant interactome revealed by coimmunoprecipitation and mass spectrometry," *Mol Plant Microbe Interact* 28:467-481 (2015), which is hereby incorporated by reference in its entirety), which were subjected to identical MS analysis, are ranked according to their abundance. Asterisk denotes MS data obtained from DeBlasio et al., "Insights into the polerovirus-plant interactome revealed by coimmunoprecipitation and mass spectrometry," *Mol Plant Microbe Interact* 28:467-481 (2015), which is hereby incorporated by reference in its entirety.

[0013] FIGS. **4**A-**4**B show interaction and PTM analysis of HSF1. FIG. **4**A shows SAINT analysis of proteins pulled-down by HSF1-GFP AptA-MS before or after HS. Dotted horizontal line represents the SAINT score cutoff (0.65). Labeled proteins above the cutoff are called as HSF1 interactors. Fold Change represents the algorithmically calculated fold change A value which takes into account representation among biological replicates. FIG. **4**B shows post-translational modifications (PTMs) on HSF1 residues identified in AptA-MS. Red and blue represent phosphorylation and acetylation, respectively. Asterisk denotes newly identified modification.

[0014] FIG. **5** shows tandem Affinity Purification of *Drosophila* HSF and its interacting partners. Lysates prepared from *Drosophila* S2 cells stably expressing NTAP-dHSF or dHSF-CTAP were subjected to Tandem Affinity Purification. Proteins in the final eluate were fractionated by SDS-PAGE and visualized by silver stain. Protein bands that were specifically co-purified with dHSF were identified by mass spectrometry in Cornell Mass Spectrometry Core Facility.

[0015] FIGS. **6**A-**6**D show MS1 and MS2 validation of post-translationally modified HSF1 peptides. As shown in FIG. **6**A, to verify post translational modifications predicted by Scaffold PTM, MS1 analysis was performed on a representative HSF1 peptide (#-GHTDTEGRPPSphosPPPTSTphosPEK-372). The normalized peak area of the modified peptide shows a consistent ratio of precursor ion masses among biological replicates regardless of condition. These precursor ion masses are derived from naturally occurring carbon isotopes C12, C13, and C14 which result in an ion with the expected mass (M) or with an addition of +1 or +2 atomic mass units (M+1, M+2). The normalized peak areas for the selected peptide are shown with M, M+1, and M+2 shown in blue, grey, and pink respectively. MS1 analysis was performed using Skyline to quantify the peak areas from a doubly phosphorylated peptide detected using AptA-MS. As shown in FIG. **6**B, the MS2 spectrum for the same modified peptide (#-GHTDTEGRPPSphosPPPTSTphosPEK-372) is consistent with the MS1 analysis and shows the expected mass shifts for S363 and T369 phosphorylation of HSF1. MS2 spectra also verify predicted HSF1 acetylation at two novel lysine (K) residues (FIG. **6**C) K62 and (FIG. **6**D) K162.

[0016] FIG. **7** shows total spectral counts over all biological replicates from histone proteins H4, H2A.Z, H2B, and H2A resulting from Mascot searches allowing for lysine acetylation (in blue), serine/threonine phosphorylation (in red), and no modification (in gray). Spectral counts are shown in both non-heat shock and heat shock conditions.

[0017] FIGS. **8**A-**8**C shows GO analysis of proteins enriched by AptA-MS. Proteins enriched by the GFP-aptamer from HSF1-GFP expressing cells compared to GFP expressing cells before or after heat shock are primarily